Study of the osteoprotegerin/receptor activator of nuclear factor-kB ligand system association with inflamm....

Article *in* Immunological investigations · January 2018

DOI: 10.1080/08820139.2017.1423499

CITATIONS
0

READS
27

7 authors, including:

Rania M. Gamal
Assiut University
13 PUBLICATIONS **35** CITATIONS
SEE PROFILE

Hanan Abozaid
Sohag University
14 PUBLICATIONS **29** CITATIONS
SEE PROFILE

Mona Sallam Embarek
Assiut University
30 PUBLICATIONS **11** CITATIONS
SEE PROFILE

Yasser Emad
Cairo University
70 PUBLICATIONS **687** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

cytokines in Rheumatoid View project

Antibodies to extractable nuclear antigens (ENAs) in RA : Do we really need to screen ? View project

Taylor & Francis
Taylor & Francis Group

Immunological Investigations

A Journal of Molecular and Cellular Immunology

ISSN: 0882-0139 (Print) 1532-4311 (Online) Journal homepage: http://www.tandfonline.com/loi/iimm20

Study of the osteoprotegerin/receptor activator of nuclear factor-kB ligand system association with inflammation and atherosclerosis in systemic sclerosis

Rania M. Gamal, Walid M. Gamal, Abeer M. Ghandour, Hanan Sayed M. Abozaid, Mona Embarek Mohamed, Yasser Emad & Ahmed Abdel Galeel

To cite this article: Rania M. Gamal, Walid M. Gamal, Abeer M. Ghandour, Hanan Sayed M. Abozaid, Mona Embarek Mohamed, Yasser Emad & Ahmed Abdel Galeel (2018): Study of the osteoprotegerin/receptor activator of nuclear factor-kB ligand system association with inflammation and atherosclerosis in systemic sclerosis, Immunological Investigations, DOI: 10.1080/08820139.2017.1423499

To link to this article: https://doi.org/10.1080/08820139.2017.1423499

Published online: 16 Jan 2018.

IMMUNOLOGICAL INVESTIGATIONS
https://doi.org/10.1080/08820139.2017.1423499

Taylor & Francis
Taylor & Francis Group

Study of the osteoprotegerin/receptor activator of nuclear factor-kB ligand system association with inflammation and atherosclerosis in systemic sclerosis

Rania M. Gamal[a], Walid M. Gamal[b], Abeer M. Ghandour[a], Hanan Sayed M. Abozaid [c], Mona Embarek Mohamed[d], Yasser Emad[e], and Ahmed Abdel Galeel[f]

[a]Rheumatology& Rehabilitation department, Assuit University, Assuit, Egypt; [b]Vascular Surgery department, Qena university hospital, South Valley University, Qena, Egypt; [c]Rheumatology& Rehabilitation department, Sohag University, Sohag, Egypt; [d]Microbiology& Immunology department, Assuit University, Assuit, Egypt; [e]Rheumatology& Rehabilitation department, Cairo University, Egypt; [f]Cardiovascular Medicine Department, Assuit University, Assuit, Egypt

ABSTRACT

Objective: we aimed to study systemic sclerosis patients in order to assess osteoprotegerin/Receptor activator of nuclear factor-kB ligand (OPG/RANKL) system and find the relation of these biomarkers with the clinical features of the disease, the carotid intima thickness, markers of inflammation, lipid profile, and other laboratory characteristics.

Methods: both the level of (RANKL), (OPG) in sera of participants, in 30 (SSc) patients and the atherosclerotic changes affecting the common carotid artery were measured and, were compared to 30 healthy controls matched for age and sex. All participants were assessed clinically and subjected to the Revised Medsger SSc severity scale and underwent carotid Doppler ultrasound examination.

Results: OPG, RANKL, and RANKL/OPG were 1.9 ± 0.4 ng/ml, 24.3 ± 17.25 ng/ml, and 13.5 ±9.8 versus 0.77 ± 0.25 ng/ml, 7.13 ± 3.02 ng/ml, and 9.6 ± 3.1 in the SSc patients and the controls with significance (P = 0.001, P = 0.001, P = 0.045) respectively. The OPG- RANKL axis in the SSc patients correlated significantly with carotid intima thickness, arthritis, arthralgia, inflammatory markers, Medsger joint, Medsger vascular, Medsger skin, and dyslipidemia.

Conclusion: In cardiovascular risks, OPG serum level might increase as a preventive compensatory mechanism to neutralize the RANKL level increment. The determination of the OPG-RANKL system is a diagnostic indicator for the intensity of vascular calcification and atherosclerosis in SSc patients.

ARTICLE HISTORY
Received 10 October 2017
Revised 28 December 2017
Accepted 30 December 2017

KEYWORDS
Atherosclerosis; carotid intima media thickness; receptor activator of nuclear factor-kB ligand System; systemic sclerosis

Introduction

Systemic Sclerosis (SSc) is a complex multisystem connective tissue disease of unknown etiology. The cardinal features of SSc are vascular injury and damage, inflammation, autoimmunity, and generalized interstitial and vascular fibrosis (Charles et al., 2006; Jimenez and Derk, 2004; Slobodin et al., 2008). The vascular calcification and atherosclerosis that occur in connective tissue diseases (including SSc) is a degenerative process (Papadopouli et al., 2008) associated with numerous regulators of bone formation and some structural proteins, for

CONTACT Rania M. Gamal drraniami@gmail.com Rheumatology and Rehabilitation department, Assuit University, Assuit, Egypt, postal code; 71515

example; Osteoprotegerin (OPG) and Receptor Activator of Nuclear Factor k Ligand (RANKL (Dhore et al., 2001). RANKL motivates osteoclast formation and survival leading to enhanced bone resorption. OPG is known as a protein homologue to Receptor Activator of Nuclear Factor kß (RANK) and can bind to RANKL, hindering the binding of RANKL to RANK, leading to suppression of differentiation of preosteoclasts to mature osteoclasts thus inhibiting osteoclastogenesis (Hofbauer and Schoppet, 2001; Souza and Lerner, 2013). There is a strong association between bone pathologies and atherosclerosis (Kiel et al., 2001), where OPG and RANKL were shown to be expressed within the atherosclerotic plaques (Dhore et al., 2001) and in a mechanism similar to that occurs during bone formation and remodeling, The OPG/RANKL/RANK axis involved in the vascular system through both immunobiologic and osteogenetic mechanisms (Papadopouli et al., 2008). Vascular calcification may involve differentiation of osteogenic cells from vascular smooth muscle cells (VSMCs) or calcifying vascular cells, with the expression of multiple ossification-related molecules, the formation of calcified structures resembling bone, and attendance of T-cells, macrophages and endothelial cells, which may form the source or target of OPG/RANKL/RANK actions. OPG looks to be a basic ligand between bone tissue and the vascular system (Hofbauer and Heufelder, 2001; Hofbauer and Schoppet, 2001) and it is produced in vessels by the endothelial cells and VSMCs (Papadopouli et al., 2008), while RANKL is not detected in normal vasculature. Small quantities of RANKL have been detected in initial stages of atherosclerosis, but the expression of RANKL was greatly increased in advanced lesions and calcified vessels and valves (Dhore et al., 2001). It is interesting that there is improvement in the survival in SSc over the past several decades, but the disease is still associated with that considerable reduction in survival when it is compared with that in age- and sex-matched populations (Abu-Shakra and Lee, 1995; Mayes et al., 2003; Medsger et al., 1971). Because the connective tissue dieases are concomitant with the increased cardiovascular morbidity and mortality, attention has been given to the presence and treatment of cardiovascular risk factors. However, to our knowledge, no data for development of accelerated atherosclerosis in the Egyptian patients with SSc as well as macrovascular risk factors assessment was available. So, we **aimed** in this study to assess the OPG/RANKL system in patients with SSc and to evaluate the correlation of these biomarkers with the clinical features of SSc, the carotid intima thickness, markers of inflammation, lipid profile, and other laboratory characteristics. Additionally, we aimed to assess the signs of atherosclerosis by measuring intima-media thickness (IMT) of the right and left common carotid artery (CCA) in both SSc patients and controls and relate the outcome to traditional risk factors.

Patients and methods

This is a cross- sectional study, included 30 SSc female patients, regularly followed up at the outpatient clinics of (Rheumatology, Rehabilitation and Physical Medicine department, Assiut University Hospital), and they were invited to participate in this study. The inclusion criteria were the following: The SSc patients fulfilled the American College of Rheumatology (ACR) classification criteria for scleroderma (Masi et al., 1980) and were further sub-classified to have diffuse (dcSSc) or limited (lcSSc) cutaneous subset of SSc according to Le Roy *et al.* (LeRoy et al., 1988), disease stages were defined as suggested by Medsger and Steen (Medsger et al., 1999): early limited SSc, disease duration < 5 years; intermediate/late limited SSc, disease duration ≥5 years; early diffuse SSc, disease duration

< 3 years; and intermediate/late SSc, disease duration ≥3 years, disease onset after 16 years of age, and use of stable medication in the last 3 months. In addition, 30 healthy volunteers were matched in age and sex to the SSc patients and served as the control group; they were recruited from the hospital workers. The exclusion criteria for the selected participants were determined as; the presence of mixed connective tissue disease and other autoimmune connective tissue pathologies overlapping with SSc, a chronic medical illness that precipitates atherosclerosis as diabetes mellitus, hypertension. Patients receiving statins, corticosteroids, or any other medications that may cause atherosclerosis or lower lipid profile was excluded from the study. Smokers, overweight, or pregnant patients were also excluded. The study was approved by the Ethical Committee of Assuit and South Valley University Hospitals. Written informed consent was obtained from all participants after they had been informed about the purpose of the study in details. All selected eligible participants were assessed clinically, and subjected to the following:- (1) Demographic data, smoking status, history and clinical examination (for SSc patients: disease duration, articular, extra-articular and organ involvements, signs of skin involvement, and treatment regimens) were recorded, (2) Revised Medsger SSc severity scale (Medsger et al., 2003) for each organ system from 0 to 4 where 0: no documented involvement or normal, 1: mild, 2: moderate, 3: severe, and 4: end stage, (3) Laboratory investigations included serum blood glucose, lipids [triglycerides (TGs), cholesterol, high- density lipoproteins (HDL) and low- density lipoproteins (LDL)], urea, and creatinine levels were measured by Hitachi 717 autoanalyzer (Roche Diagnostics) routinely used in the hospital laboratory. Hemoglobin (HB) level, C-reactive protein (CRP)-(CTM- USA), and erythrocyte sedimentation rate (ESR) were also determined. Biochemical markers; OPG, and RANKL levels were measured in patients' sera by ELISA using commercially available kits (R&D Systems, UK) depended on manufacturer's instructions. Blood samples for ELISA were centrifuged, and sera stored at 20°C for ≤ 1 month until used. All participants underwent carotid Doppler Ultrasound examination using Philips HDI 5000 duplex with a 7.5–12 megahertz linear array, the IMT of CCA was measured 1 cm distal to the carotid bifurcation in the posterior wall. For each patient, the highest IMT among the measured segments studied on each side was recorded. According to current sonographic criteria, IMT was considered "normal" when less than 0.9 mm, "thickened" when IMT was equal to or more than 0.9 mm, and when the thickness was more than 1.3 mm was indicative of atherosclerotic plaque (Doria et al., 2003).

Statistical analysis: Data analyzed by SPSS version 20.0 statistical package, data were presented as number and percent, mean ±SD, or median and range as appropriate. Student's *t*-test and multivariate analysis were used for comparing means between different groups, Pearson correlation coefficient (*r*) was used to test the association between quantitative variables. A *p*-value less than 0.05 was considered statistically significant.

Results

Characteristics of the study population

The Controls and the SSc patients were matched for age, sex, and smoking status. All our SSc patients, and controls were females, and e non-smokers. Abnormal ECG findings, CCA atheromas, and history of drug therapy were present in SSc patients only. CCA.IMT

was significantly higher in SSc patients than controls (Table 1). CRP and proteinuria was positive in 20 (66.7%) and 12 (40%) SSc patients respectively, and negative for all controls (χ2; P = 0.000). ESR was high in 27 (90%) SSc patients versus 2 (6.7%) controls (χ2; P = 0.000). Creatinine clearance was abnormal in only 2 (6.7%) SSc patients and normal for the control group (χ2; P = 0.15). Demographic data, laboratory data, and severity score index of SSc patients are summarized in Table 2.

OPG–RANKL axis was significantly higher in SSc patients compared to controls

OPG levels were 1.9 ± 0.4 (mean ± SD) ng/ml in SSc patients versus 0.77 ± 0.25 ng/ml in controls, with (P = 0.001), RANKL levels were 24.3 ± 17.25 ng/ml in SSc patients versus 7.13 ± 3.02 ng/ml in controls, with (P = 0.001). While sRANKL/OPG ratios were 13.5 ± 9.8 versus 9.6 ± 3.1 in SSc patients and controls, with (P = 0.045), (Figure 1).

Levels of OPG, srankl and, RANKL/OPG and clinical findings in ssc patients

Univariate analysis showed that, in SSc patients, OPG levels were significantly higher in those with active ulcers (P = 0.035). OPGs, RANKL, and sRANKL/OPG were significantly higher in patients with late SSc than those with early SSc (P = 0.001) for each of them, and in patients with acrolysis (P = 0.018, 0.001, and 0.022, respectively). Patients with telangiectasia exhibited significantly higher levels of OPG and RANKL compared with their counter parts (P = 0.001 and 0.001, respectively). Both RANKL and RANKL/OPG were higher in SSc patients with contracture (P = 0.001 and 0.001, respectively) and myalgia (P = 0.001 and 0.003, respectively). OPGs, RANKL, and sRANKL/OPG were positively correlated with the number of joints affected by arthralgia and arthritis, Medsger joint, Medsger skin, while OPG only correlated significantly to Medsger vascular

Table 1. investigatory findings and risk factors among studies SSc cases and control groups.

	SSc group (n = 30)	Control group (n = 30)	*P* .values
₮ Age	37.1 ± 8.5	36.9 ± 7	0.96
ECG findings	19(63%)	30(100%)	**0.009**
Normal	1(3.3%)		
Tachycardia	4(13.4%)		
Ventricular hypertrophy	3 (10%)		
Arrhythmia	3(10%)		
Ischemic changes			
₮ Right.CCA.IMT (mm)	1.07 ± 0.56	0.6 ± 0.11	**0.000**
₮ Left.CCA.IMT (mm)	0.98 ± 0.44	0.64 ± 0.096	**0.000**
Intimae thickness ≥0.9mm	1.07 ± 0.56	0.6 ± 0.11	**0.000***
₮ Right CCA Atheroma	0.98 ± 0.44	0.64 ± 0.096	**0.000***
₮ Left CCA Atheroma			
Cr .clearance	2(6.7%)	0(0%)	0.15
Proteinuria	12(40%)	0(0%)	0.000*
positive CRP (mg/dl)	20(66.7%)	0(0%)	0.000*
High ESR 1st hour (mm/h)	27(90%)	2(6.7%)	**0.000***
Drug therapy	18(60%)	0(0%)	**0.000**
Steroid intake			
Statins intake	7(23.3%)	0(0%)	**0.004**
Family history of CV disease	11(36.7%)	8 (26.7%)	0.405

₮,Data are expressed as mean ± SD others data are number (%) as appropriate; CCA, Common Carotid Artery (CCA); IMT, Intima-Media Thickness; ESR, Erythrocyte Sedimentation Rate; CRP, C-reactive protein; Cr, Creatinine; CV, Cardiovascular.

Table 2. Clinical characteristics, laboratory features and severity score index of SSc patients (n = 30).

General Characteristics	Mean ± SD or n (%) (range)
₮ Age	37.0 ± 8.47
₮ Disease duration(years)	8.3 ± 6.6
Early SSc (≤ 3 years)	8 (26.7%)
Late SSc (≥ 10 years)	9 (30%)
₮ Modified Rodman's score	18.9 ± 7.3
₮ Raynaud's Duration	18. ± 10.6
₮ Raynaud's Frequency	2.57 ± 1.12
Digital Ulcers	11 (36.7%)
Pitting scars	29 (96.7%)
Calcinosis	1 (3.33%)
Telangictasia	8 (26.7)
Gangrene	1 (3.33%)
₮ Swollen joint count	2.56 ± 3.758
₮ Painful joint count	4.72 ± 3.855
Contracture	15 (50%)
Acrolysis	14 (46.7%)
Right hand closure	2.6 ± 1.89
Left hand closure	2.43 ± 2
Mouth opening	3.17 ± 0.89 (2–6)
Weakness	2 (6.7%)
Myalgia	11 (36.7%)
Laboratory Investigations	
₮ Hemoglobin(HB)(gm/dl)	11.88 ± 0.93
₮ ESR(mm/1st hour)	30.2 ± 22
₮ C. reactive protein titer (CRP) (mg/dl)	31 ± 30.4
₮ S. Creatinine (µmol/l)	58 ± 12.5
₮ RBS (mmol/l)	5 ± 1.2
₮ Cholesterol(mg/dl)	178.5 ± 38.8
₮ HDL (mg/dl)	43.7 ± 9
₮ LDL(mg/dl)	108.14 ± 25.8
TG (mg/dl)	116.11 ± 49.6
Severity score index	
₮ General	0.74 ± 0.74
₮ Peripheral vascular	2.3 ± 0.7
₮ Skin	2 ± 0.7
₮ Joint/tendon	1.24 ± 0.94
₮ Muscle	0.31 ± 0.46
₮ Pulmonary	0.62 ± 0.94
₮ Cardiac	0.1 ± 0.34
	0.36 ± 1.13

₮ Data are expressed as mean ± SD others data are number (%) as appropriate; ESR, Erythrocyte Sedimentation Rate; CRP, C-reactive protein;RBS, Random Blood Sugar; HDL, High Density Lipoprotein; LDL,Low density Lipoprotein;TG,Triglycerides.

(Table 3). Otherwise, no significant differences were detected in OPG, RANKL, and sRANKL/OPG in SSc patients regarding other clinical findings (P > 0.05).

Levels of OPG, srankl and, RANKL/OPG and laboratory and radiological findings in ssc patients

By univariate regression, we have found both right and left maximum IMT were positively correlated to the OPG/RANKL axis. But with the multiple regression analysis, including other factors that may affect IMT like age; body mass index (BMI); blood sugar level; blood pressure; disease duration; levels of triglycerides; HDL; LDL; and cholesterol, right maximum IMT was significantly correlated with OPG (P = 0.046) and to a lesser extent with the disease

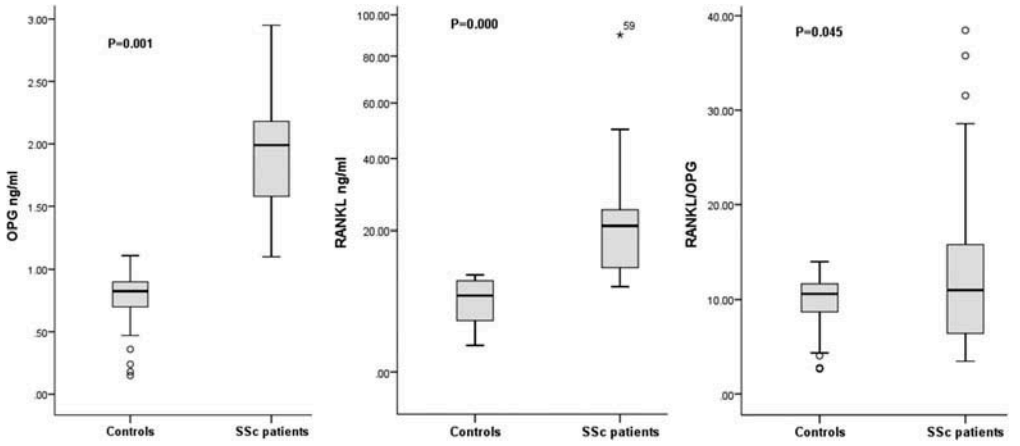

Figure 1. Serum levels of biomarkers were significantly higher in SSc patients compared to controls.

Table 3. Correlation between biological markers and carotid, clinical, and laboratory parameters in SSc patients.

	sOPG concentration		sRANKL concentration		RANKL/OPG	
	CC	*P*. value	CC	*P* .value	CC	*P* .value
RT.CCA.IMT	**0.493**	**0.006***	**0.374**	**0.042***	**0.333**	**0.072**
LT.CCA.IMT	**0.579**	**0.001***	**0.506**	**0.004***	**0.457**	**0.011***
Arthritis	**0.578**	**0.001***	**0.424**	**0.020***	**0.263**	**0.161**
Arthralgia	**0.474**	**0.008**	**0.703**	**0.000****	**0.417**	**0.022***
Medsger joint/tendon	**0.431**	**0.017**	**0.571**	**0.001***	**0.428**	**0.018***
Medsger skin score	**0.377**	**0.040***	**0.515**	**0.004***	**0.395**	**0.031***
Medsger vascular score	**0.489**	**0.006***	**0.353**	**0.055***	**0.197**	**0.297**
ESR	**0.547**	**0.002***	**0.929**	**0.000***	**0.491**	**0.006***
CRP	**0.531**	**0.003***	**0.408**	**0.025***	**0.180**	**0.340**
LDL	**0.451**	**0.012***	**0.969**	**0.000***	**0.475**	**0.008***
TG	**0.234**	**0.213**	**0.586**	**0.001***	**0.524**	**0.003***
Cholesterol	**0.304**	**0.103**	**0.443**	**0.014***	**0.207**	**0.272**

*Statistically significant value (P < 0.05); CCA, Common Carotid Artery (CCA); IMT, Intima-Media Thickness; ESR, Erythrocyte Sedimentation Rate; CRP, C-reactive protein; RBS, Random Blood Sugar; HDL, High Density Lipoprotein; LDL, Low density Lipoprotein; TG, Triglycerides.

duration and blood glucose levels (P = 0.054 and 0.00.059, respectively). Left IMT correlated significantly with OPG and RANKL levels (P = 0.029 and 0.032, respectively), blood glucose levels (P = 0.009), and to a lesser extent with age (P = 0.092). Markers of inflammation, abnormal lipid profile were also correlated significantly with OPG/RANKL axis (Table 3).

Discussion

Although the microvasculature is affected primarily in SSc, the affection of large vessels may also found in 50–60% of SSc patients (Ho et al., 2000; LeRoy, 1996; Matsuura et al., 2009; Shoenfeld et al., 2005).The tissue damage in macrovascular disease is caused by large vessel occlusion and transmural inflammation of the vessel wall, the assessment of CCA.IMT is an indicator of atherosclerosis, and it is found to be affected in 64% of SSc patients (LeRoy, 1996). So, we evaluated the process of atherosclerosis in SSc patients, and correlated it to

vascular biomarkers. Our data revealed that SSc patients had significantly greater carotid IMT than controls suggesting an increased prevalence of early atherosclerotic macrovascular disease in SSc. This was in agreement with previous works that reported a significant increase in IMT of CCA in SSc patients compared with controls (Au et al., 2011; Tsifetaki et al., 2010). In contrast to our findings, other studies (Cheng et al., 2003; Motegi et al., 2014) found that there was no significant increase in the mean CCA.IMT in SSc patients compared with healthy controls. This discrepancy may be explained by the larger percentage of the diffuse cutaneous systemic sclerosis (dcSSc) subtype that present in these studies compared to our study, and may be also due to different clinical or ethnic data. Additionally, we reported the presence of carotid atheromas in SSc patients not in controls, in accord with former findings (Au et al., 2011; Ho et al., 2000). In our study, The OPG, RANKL, and RANKL/OPG in SSc subjects were higher than in healthy controls as previously reported (Dovio et al., 2008) and the right IMT was significantly correlated with the OPG levels, while the left IMT was significantly correlated with the OPG and RANKL levels, suggesting their key role in vascular calcification. Moreover, serum OPG levels in this work correlated significantly with the Medsger vascular in SSc patients. OPG and RANKL were detected previously in the atherosclerotic plaques (Schoppet et al., 2004) and Kiechl *et al.* (Kiechl et al., 2007) stated that serum RANKL has been associated with increased risk of cardiovascular disease. Our results were compatible with the findings of Breland *et al.* (Breland et al., 2010) who could explain the important role of the OPG-RANKL system in the pathogenesis of atherosclerosis in inflammatory rheumatic diseases including SSc.

Some clinical studies have shown high serum OPG levels related to severity, and progression of coronary artery disease, endothelial dysfunction, and smooth cell hypertrophy, or advanced plaque calcifications (Golledge et al., 2004). It was shown also that human carotid atherosclerotic lesions presented elevated OPG and RANKL immunoreactivity (Golledge et al., 2004; Schoppet et al., 2004). The different effect of OPG depends on the stage of the atherosclerotic lesion. In early stages, OPG may be increased to protect vessels, by activating inflammatory pathways, to compensate vasculature damage. As the lesion progresses, OPG may become injurious to the vessels or fail to reverse the procedure of calcification (Nybo and Rasmussen, 2008; Papadopouli et al., 2008; Van Campenhout and Golledge, 2009; Vega et al., 2007; Venuraju et al., 2010). The right IMT to some extends correlated with disease duration. Previous reports attributed these relations by reduced numbers of bone marrow-derived circulating endothelial progenitor cells (EPCs) late in SSc. EPCs enhance vascular healing by homing in the damaged endothelium. The reduced level of circulating EPCs seems to define a more active disease, with the risk of digital vascular lesions and higher severity score (Cannarile et al., 2015). Although did not reach a significant level, the left IMT correlated with a lesser extend with age of patients. The age-related changes in vascular structures include degeneration and sclerosis of the vascular media; and intimal changes associated with atherosclerosis that start early in life and progress with aging, resulting in arteriosclerosis with decreased arterial elasticity, increased collagen, and calcification (Sawabe, 2010). Our OPG/RANKL axis showed a significant higher level in patients with late SSc than those with early SSc. OPG and RANKL concentrations have been higher in advanced human atherosclerotic lesions, with OPG situated at the margins of mineralized lamellar bone-like structures, RANKL associated with the adjoining matrix (Collin-Osdoby, 2004). OPG levels in our work were significantly higher in SSc patients with active ulcers. Both OPG and RANKL showed a relation with skin ulcers consonant with their reported in-vitro functional effects on

endothelial cells (ECs) (Dovio et al., 2008). Our OPG-RANKL system correlated significantly with the degree of arthritis and arthralgia, and Medsger joint/tendon in SSc patients. RANKL expression was detected at sites of active bone erosions in arthritis which explains that bone erosions are the outcome of osteoclastic bone resorption at the sites of synovitis (Pettit et al., 2006). The highest degree of radiographic progression of joint damage also was found with a combination of high ESR and high RANKL/OPG ratio at baseline which indicate that the combination of the degree of inflammation and bone destruction is a determinant of arthritis progression(Geusens, 2012). In our work, the OPG-RANKL axis correlated with inflammatory markers in SSc patients. CRP is a strong predictor of cardiovascular disease, and atherosclerosis, it is one of the pathogenic factors involved in SSc-associated vascular damage (Herrick and Cerinic, 2001; Shoenfeld et al., 2005). We found associations between OPG and RANKL, and their ratio (RANKL/OPG) and serum lipids in this study. Hyperlipidemia is independently associated with circulating levels of RANKL and/or OPG and the interplay of lipids with the RANKL-OPG axis may have a role in the progression of atherosclerosis (Kelesidis et al., 2013) as it rises the risk of retention and modification of low-density lipoprotein (LDL) in arterial walls, increased LDL is another pathogenic factor involved in SSc-associated vascular damage (Herrick and Cerinic, 2001; Shoenfeld et al., 2005). As RANKL, and OPG are members of one system and strongly associated, previous studies have conflicting results. Some studies reported the increased serum level of OPG in coronary artery calcification, while there is other evidence of inhibitory effects of OPG on vascular calcification (Falk, 2006). Therefore, our study determined the changes in RANKL and OPG levels concomitantly and reported the RANKL/OPG ratio in SSc patients with atherosclerosis.

Possible limitations of our study include the cross-sectional study design, lack of a male control group, and the small number of the participants. from our study findings, it is concluded that, in cardiovascular events, OPG serum level may increase to neutralize the RANKL level increment. RANKL levels might be affected by factors other than OPG which need further investigation with larger sample size to better clarify their role as a diagnostic indicator for the intensity of vascular calcification and atherosclerosis in SSc patients.

Highlights in the article

- Systemic Sclerosis effect on macrovascular.
- Bone pathologies and their link with atherosclerosis.
- OPG-RANKL correlation with Carotid Artery Intima Media Thickness.

Declaration of interest

The authors declare that there is no conflict of interest.

Funding

The authors did not receive any financial fund support or grants.

ORCID

Hanan Sayed M. Abozaid http://orcid.org/0000-0003-1601-2578

References

Abu-Shakra M, Lee P. 1995. Mortality in systemic sclerosis: A comparison with the general population. J Rheumatol, 22, 2100–2102

Au K, Singh MK, Bodukam V, et al. 2011. Atherosclerosis in systemic sclerosis: A systematic review and meta-analysis. Arthritis Rheum, 63, 2078–2090

Breland UM, Hollan I, Saatvedt K, et al. 2010. Inflammatory markers in patients with coronary artery disease with and without inflammatory rheumatic disease. Rheumatology, 49, 1118–1127

Cannarile F, Valentini V, Mirabelli G, et al. 2015. Cardiovascular disease in systemic sclerosis. Annals of Translational Medicine, 3, 8

Charles C, Clements P, Furst DE. 2006. Systemic sclerosis: hypothesis-driven treatment strategies. The Lancet, 367, 1683–1691

Cheng K-S, Tiwari A, Boutin A, et al. 2003. Carotid and femoral arterial wall mechanics in scleroderma. Rheumatology, 42, 1299–1305

Collin-Osdoby P. 2004. Regulation of vascular calcification by osteoclast regulatory factors RANKL and osteoprotegerin. Circ Res, 95, 1046–1057

Dhore CR, Cleutjens JP, Lutgens E, et al. 2001. Differential expression of bone matrix regulatory proteins in human atherosclerotic plaques. Arterioscler Thromb Vasc Biol, 21, 1998–2003

Doria A, Shoenfeld Y, Wu R, et al. 2003. Risk factors for subclinical atherosclerosis in a prospective cohort of patients with systemic lupus erythematosus. Ann Rheum Dis, 62, 1071–1077

Dovio A, Data V, Carignola R, et al. 2008. Circulating osteoprotegerin and soluble RANK ligand in systemic sclerosis. J Rheumatol, 35, 2206–2213

Falk E. 2006. Pathogenesis of atherosclerosis. J Am Coll Cardiol, 47, C7–C12

Geusens P. 2012. The role of RANK ligand/osteoprotegerin in rheumatoid arthritis. Ther Adv Musculoskelet Dis, 4, 225–233

Golledge J, McCann M, Mangan S, et al. 2004. Osteoprotegerin and osteopontin are expressed at high concentrations within symptomatic carotid atherosclerosis. Stroke, 35, 1636–1641

Herrick A, Cerinic MM. 2001. The emerging problem of oxidative stress and the role of antioxidants in systemic sclerosis. Clin Exp Rheumatol, 19, 4–8

Ho M, Veale D, Eastmond C, et al. 2000. Macrovascular disease and systemic sclerosis. Ann Rheum Dis, 59, 39–43

Hofbauer LC, Heufelder AE. 2001. Role of receptor activator of nuclear factor-κB ligand and osteoprotegerin in bone cell biology. J Mol Med, 79, 243–253

Hofbauer LC, Schoppet M. 2001. Osteoprotegerin: A link between osteoporosis and arterial calcification? The Lancet, 358, 257–259

Jimenez SA, Derk CT. 2004. Following the molecular pathways toward an understanding of the pathogenesis of systemic sclerosis. Ann Intern Med, 140, 37–50

Kelesidis T, Kendall MA, Yang OO, et al. 2013. Perturbations of circulating levels of RANKL-osteoprotegerin axis in relation to lipids and progression of atherosclerosis in HIV-infected and-uninfected adults: ACTG NWCS 332/A5078 Study. AIDS Res Hum Retroviruses, 29, 938–948

Kiechl S, Schett G, Schwaiger J, et al. 2007. Soluble receptor activator of nuclear factor-κB ligand and risk for cardiovascular disease. Circulation, 116, 385–391

Kiel DP, Kauppila LI, Cupples LA, et al. 2001. Bone loss and the progression of abdominal aortic calcification over a 25 year period: The Framingham Heart Study. Calcif Tissue Int, 68, 271–276

LeRoy EC. 1996. Systemic sclerosis: A vascular perspective. Rheum Dis Clin America, 22, 675–694

LeRoy EC, Black C, Fleischmajer R, et al. 1988. Scleroderma (systemic sclerosis): Classification, subsets and pathogenesis. J Rheumatol, 15, 202

Masi AT, Rodnan G, Medsger Jr T, et al. 1980. Subcommittee for scleroderma criteria of the American rheumatism association diagnostic and therapeutic criteria committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum, 23, 581–590

Matsuura E, Kobayashi K, Lopez LR. 2009. Atherosclerosis in autoimmune diseases. Curr Rheumatol Rep, 11, 61–69

Mayes MD, Lacey JV, Beebe-Dimmer J, et al. 2003. Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. Arthritis Rheum, 48, 2246–2255

Medsger T, Bombardieri S, Czirjak L, et al. 2003. Assessment of disease severity and prognosis. Clin Exp Rheumatol, 21, S42–S46

Medsger TA, Masi AT, Rodnan GP, et al. 1971. Survival with systemic sclerosis (scleroderma): a life-table analysis of clinical and demographic factors in 309 patients. Ann Intern Med, 75, 369–376

Medsger TA, Silman AJ, Steen VD, et al. 1999. A disease severity scale for systemic sclerosis: development and testing. J Rheumatol, 26, 2159–2167.

Motegi S, Toki S, Hattori T, et al. 2014. No association of atherosclerosis with digital ulcers in Japanese patients with systemic sclerosis: Evaluation of carotid intima-media thickness and plaque characteristics. J Dermatol, 41, 604–608

Nybo M, Rasmussen LM. 2008. The capability of plasma osteoprotegerin as a predictor of cardiovascular disease: A systematic literature review. European Journal of Endocrinology, 159, 603–608

Papadopouli AE, Klonaris CN, Theocharis SE. 2008. Role of OPG-RANKL-RANK axis on the vasculature. Histol Histopathol, 23, 497–506.

Pettit AR, Walsh NC, Manning C, et al. 2006. RANKL protein is expressed at the pannus-bone interface at sites of articular bone erosion in rheumatoid arthritis. *Rheumatology (Oxford)*, 45, 1068–1076

Sawabe M. 2010. Vascular aging: From molecular mechanism to clinical significance. Geriatr Gerontol Int, 10, S213–S220

Schoppet M, Al-Fakhri N, Franke FE, et al. 2004. Localization of osteoprotegerin, tumor necrosis factor-related apoptosis-inducing ligand, and receptor activator of nuclear factor-κB ligand in Monckeberg's sclerosis and atherosclerosis. J Clin Endocrinol Metabolism, 89, 4104–4112

Shoenfeld Y, Gerli R, Doria A, et al. 2005. Accelerated atherosclerosis in autoimmune rheumatic diseases. Circulation, 112, 3337–3347

Slobodin G, Pavlotzky E, Panov J, et al. 2008. Endothelin-1 does not change the function of monocyte-derived dendritic cells grown from patients with systemic sclerosis. Immunol Invest, 37, 841–848

Souza PPC, Lerner UH. 2013. The role of cytokines in inflammatory bone loss. Immunol Invest, 42, 555–622

Tsifetaki N, Georgiadis A, Alamanos Y, et al. 2010. Subclinical atherosclerosis in scleroderma patients. Scand J Rheumatol, 39, 326–329

Van Campenhout A, Golledge J. 2009. Osteoprotegerin, vascular calcification and atherosclerosis. Atherosclerosis, 204, 321–329

Vega D, Maalouf NM, Sakhaee K. 2007. CLINICAL Review #: The role of receptor activator of nuclear factor-kappaB (RANK)/RANK ligand/osteoprotegerin: clinical implications. J Clin Endocrinol Metab, 92, 4514–4521

Venuraju SM, Yerramasu A, Corder R, Lahiri A. 2010. Osteoprotegerin as a predictor of coronary artery disease and cardiovascular mortality and morbidity. J Am Coll Cardiol, 55, 2049–2061